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(54) **PEPTIDE FRAGMENTS FOR INDUCING SYNTHESIS OF EXTRACELLULAR MATRIX PROTEINS**

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(57) **ABSTRACT**

Short biologically active tetrapeptides are disclosed that are comprised of the sequences GxxG and PxxP where G (glycine) and P (proline) are maintained and x is a variable amino acid. The peptides can be used singly or in combination to stimulate production of extracellular matrix proteins in skin. A rapid, low-cost method of producing heterogenous formulations of tetrapeptides is disclosed.

14 Claims, 3 Drawing Sheets

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MGPRLSVWLL LLPAALLLHE EHSRAAAKGG CAGSGCGKCD CHGVKGQKGE
RGLPGLQGVI GFPGMQGPEG PQGPPGQKGD TGEPGLPGTK GTRGPPGASG
YPGNPGLPGI PGQDGPPGPP GIPGCNGTKG ERGPLGPPGL PGFAGNPGPP
GLPGMKGDPG EILGHVPGML LKGERGFPGI PGTPGPPGLP GLQGPVGPPG
FTGPPGPPGP PGPPGEKGQM GLSFQGPKGD KGDQGVSGPP GVPGQAQVQE
KGDFATKGEK GQKGEPGFQG MPGVGEKGEP GKPGPRGKPG KDGDKGEKGS
PGFPGEPGYP GLIGRQGPQG EKGEAGPPGP PGIVIGTGPL GEKGERGYPG
TPGPRGEPGP KGFPGLPGQP GPPGLPVPGQ AGAPGFPGER GEKGDRGFPG
TSLPGPSGRD GLPGPPGSPG PPGQPGYTNG IVECQPGPPG DQGPPGIPGQ
PGFIGEIGEK GQKGESCLIC DIDGYRGPPG PQGPPGEIGF PGQPGAKGDR
GLPGRDGVAG VPGPQGTPGL IGQPGAKGEP GEFYFDLRLK GDKGDPGFPG
QPGMPGRAGS PGRDGHPGLP GPKGSPGSVG LKGERGPPGG VGFPGSRGDT
GPPGPPGYGP AGPIGDKGQA GFPGGPGSPG LPGPKGEPGK IVPLPGPPGA
EGLPGSPGFP GPQGDRGFPG TPGRPGLPGE KGAVGQPGIG FPGPPGPKGV
DGLPGDMGPP GTPGRPGFNG LPGNPGVQGQ KGEPGVGLPG LKGLPGLPGI
PGTPGEKGSI GVPGVPGEHG AIGPPGLQGI RGEPGPPGLP GSVGSPGVPG
IGPPGARGPP GGQGPPGLSG PPGIKGEKGF PGFPGLDMPG PKGDKGAQGL
PGITGQSGLP GLPGQQGAPG IPGFPGSKGE MGVMGTPGQP GSPGPVGAPG
LPGEKGDHGF PGSSGPRGDP GLKGDKGDVG LPGKPGSMDK VDMGSMKGQK
GDQGEKGQIG PIGEKGSRGD PGTPGVPGKD GQAGQPGQPG PKGDPGISGT
PGAPGLPGPK GSVGGMGLPG TPGEKGVPGI PGPQGSPGLP GDKGAKGEKG
QAGPPGIGIP GLRGEKGDQG IAGFPGSPGE KGEKGSIGIP GMPGSPGLKG
SPGSVGYPGS PGLPGEKGDK GLPGLDGIPG VKGEAGLPGT PGPTGPAGQK
GEPGSDGIPG SAGEKGEPGL PGRGFPGFPG AKGDKGSKGE VGFPGLAGSP
GIPGSKGEQG FMGPPGPQGQ PGLPGSPGHA TEGPKGDRGP QGQPGLPGLP
GPMGPPGLPG IDGVKGDKGN PGWPGAPGVP GPKGDPGFQG MPGIGGSPGI
TGSKGDMGPP GVPGFQGPKG LPGLQGIKGD QGDQGVPGAK GLPGPPGPPG
PYDIIKGEPG LPGPEGPPGL KGLQGLPGPK GQQGVTGLVG IPGPPGIPGF
DGAPGQKGEM GPAGPTGPRG FPGPPGPDGL PGSMGPPGTP SVDHGFLVTR
HSQTIDDPQC PSGTKILYHG YSLLYVQGNE RAHGQDLGTA GSCLRKFSTM
PFLFCNINNV CNFASRNDYS YWLSTPEPMP MSMAPITGEN IRPFISRCAV
CEAPAMVMAV HSQTIQIPPC PSGWSSLWIG YSFVMHTSAG AEGSGQALAS
PGSCLEEFRS APFIECHGRG TCNYYANAYS FWLATIERSE MFKKPTPSTL
KAGELRTHVS RCQVCMRRT

FIG. 1

MMSFVQKGSW LLLALLHPTI ILAQQEAVEG GCSHLGQSYA DRDVWKPEPC
QICVCDSGSV LCDDIICDDQ ELDCPNPEIP FGECCAVCPQ PPTAPTRPPN
GQGPQGPKGD PGPPGIPGRN GDPGIPGQPG SPGSPGPPGI CESCPTGPQN
YSPQYDSYDV KSGVAVGGLA GYPGPAGPPG PPGPPGTSGH PGSPGSPGYQ
GPPGEPGQAG PSGPPGPPGA IGPSGPAGKD GESGRPGRPG ERGLPGPPGI
KGPAGIPGFP GMKGHRGFDG RNGEKGETGA PGLKGENGLP GENGAPGPMG
PRGAPGERGR PGLPGAAGAR GNDGARGSDG QPGPPGPPGT AGFPGSPGAK
GEVGPAGSPG SNGAPGQRGE PGPQGHAGAQ GPPGPPGING SPGGKGEMGP
AGIPGAPGLM GARGPPGPAG ANGAPGLRGG AGEPGKNGAK GE**PGPR**GERG
EAGIPGVPGA KGEDGKDGSP GEPGANGLPG AAGERGAPGF RGPAGPNGIP
GEKGPAGERG APGPAGPRGA AGEPGRDGVP GGPGMRGMPG SPGGPGSDGK
PGPPGSQGES GRPGPPGPSG PRGQPGVMGF PGPKGNDGAP GKNGERGGPG
GPGPQGPPGK NGETGPQGPP GPTGPGGDKG DTGPPGPQGL QGLPGTGGPP
GENGKPGEPG PKGDAGAPGA PGGKGDAGAP GERGPPGLAG APGLRG**GAGP**
PGPEGGKGAA GPPGPPGAAG TPGLQGMPGE RGGLGSPGPK GDKGEPGGPG
ADGVPGKDGP RGPTGPIGPP GPAGQPGDKG EGGAPGLPGI AGPRGSPGER
GETGPPGPAG FPGAPGQNGE PGGKGERGAP GEKGEGGPPG VAGPPGGSGP
AGPPGPQGVK GERGSPGGPG AAGFPGARGL PGPPGSNGNP GPPGPSGSPG
KDGPPGPAGN TGAPGSPGVS GPKGDAGQPG EKGSPGAQGP PGAPGPLGIA
GITGARGLAG PPGM**PGPR**GS PGPQGVKGES GKPGANGLSG ERGPPGPQGL
PGLAGTAGEP GRDGNPGSDG LPGRDGSPGG KGDRGENGSP GAPGAPGHPG
PPGPVGPAGK SGDRGESGPA GPAGAPGPAG SRGAPGPQGP RGDKGETGER
GAAGIKGHRG FPGNPGAPGS PGPAGQQGAI GSPGPAGPRG PVGPSGPPGK
DGTSGHPGPI GP**PGPR**GNRG ERGSEGSPGH PGQPGPPGPP GAPGPCCGGV
GAAAIAGIGG EKAGGFAPYY GDEPMDFKIN TDEIMTSLKS VNGQIESLIS
PDGSRKNPAR NCRDLKFCHP ELKSGEYWVD PNQGCKLDAI KVFCNMETGE
TCISANPLNV PRKHWWTDSS AEKKHVWFGE SMDGGFQFSY GNPELPEDVL
DVQLAFLRLL SSRASQNITY HCKNSIAYMD QASGNVKKAL KLMGSNEGEF
KAEGNSKFTY TVLEDGCTKH TGEWSKTVFE YRTRKAVRLP IVDIAPYDIG
GPDQEFGVDV GPVCFL

FIG. 2

MGPRLSVWLL LLPAALLLHE EHSRAAAKGG CAGSGCGKCD CHGVKGQKGE
RGLPGLQGVI GFPGMQGPEG PQGPPGQKGD TGEPGLPGTK GTRGPPGASG
YPGNPGLPGI PGQDGP**PGPP** GIPGCNGTKG ERGPLGPPGL PGFAGN**PGPP**
GLPGMKGDPG EILGHVPGML LKGERGFPGI PGT**PGPP**GLP GLQGPVGPPG
FTGP**PGPP**GP **PGPP**GEKGQM GLSFQGPKGD KGDQGVSGPP GVPGQAQVQE
KGDFATKGEK GQKGEPGFQG MPGVGEKGEP GKPGPRGKPG KDGDKGEKGS
PGFPGEPGYP GLIGRQGPQG EKGEAGP**PGP P**GIVIGTGPL GEKGERGYPG
TPGPRGEPGP KGFPGLPGQP GPPGLPVPGQ AGAPGFPGER GEKGDRGFPG
TSLPGPSGRD GL**PGPP**GSPG PPGQPGYTNG IVECQ**PGPP**G DQGPPGIPGQ
PGFIGEIGEK GQKGESCLIC DIDGYRGPPG PQGPPGEIGF PGQPGAKGDR
GLPGRDGVAG VPGPQGTPGL IGQPGAKGEP GEFYFDLRLK GDKGDPGFPG
QPGMPGRAGS PGRDGHPGLP GPKGSPGSVG LKGERGPPGG VGFPGSRGDT
GP**PGPP**GYGP AGPIGDKGQA GFPGGPGSPG LPGPKGEPGK IVPL**PGPP**GA
EGLPGSPGFP GPQGDRGFPG TPGRPGLPGE KGAVGQPGIG F**PGPP**GPKGV
DGLPGDMGPP GTPGRPGFNG LPGNPGVQGQ KGEPGVGLPG LKGLPGLPGI
PGTPGEKGSI GVPGVPGEHG AIGPPGLQGI RGE**PGPP**GLP GSVGSPGVPG
IGPPGARGPP GGQGPPGLSG PPGIKGEKGF PGFPGLDMPG PKGDKGAQGL
PGITGQSGLP GLPGQQGAPG IPGFPGSKGE MGVMGTPGQP GSPGPVGAPG
LPGEKGDHGF PGSSGPRGDP GLKGDKGDVG LPGKPGSMDK VDMGSMKGQK
GDQGEKGQIG PIGEKGSRGD PGTPGVPGKD GQAGQPGQPG PKGDPGISGT
PGAPGLPGPK GSVGGMGLPG TPGEKGVPGI PGPQGSPGLP GDKGAKGEKG
QAGPPGIGIP GLRGEKGDQG IAGFPGSPGE KGEKGSIGIP GMPGSPGLKG
SPGSVGYPGS PGLPGEKGDK GLPGLDGIPG VKGEAGLPGT PGPTGPAGQK
GEPGSDGIPG SAGEKGEPGL PGRGFPGFPG AKGDKGSKGE VGFPGLAGSP
GIPGSKGEQG FMGPPGPQGQ PGLPGSPGHA TEGPKGDRGP QGQPGLPGLP
GPMGPPGLPG IDGVKGDKGN PGWPGAPGVP GPKGDPGFQG MPGIGGSPGI
TGSKGDMGPP GVPGFQGPKG LPGLQGIKGD QGDQGVPGAK GL**PGPP**GPPG
PYDIIKGEPG LPGPEGPPGL KGLQGLPGPK GQQGVTGLVG I**PGPP**GIPGF
DGAPGQKGEM GPAGPTGPRG F**PGPP**GPDGL PGSMGPPGTP SVDHGFLVTR
HSQTIDDPQC PSGTKILYHG YSLLYVQGNE RAHGQDLGTA GSCLRKFSTM
PFLFCNINNV CNFASRNDYS YWLSTPEPMP MSMAPITGEN IRPFISRCAV
CEAPAMVMAV HSQTIQIPPC PSGWSSLWIG YSFVMHTSAG AEGSGQALAS
PGSCLEEFRS APFIECHGRG TCNYYANAYS FWLATIERSE MFKKPTPSTL
KAGELRTHVS RCQVCMRRT

FIG. 3

PEPTIDE FRAGMENTS FOR INDUCING SYNTHESIS OF EXTRACELLULAR MATRIX PROTEINS

This application is a continuation of application Ser. No. 11/811,876 filed Jun. 12, 2007, now U.S. Pat. No. 8,110,658, which claims benefit of priority to U.S. 60/813,284 filed Jun. 13, 2006, each of which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The invention relates to tetrapeptides with the amino acid motif GxxG or PxxP, where G (glycine) and P (proline) are maintained and x is a variable amino acid. The invention also relates to frame shift active tetrapeptides which are tetrapeptide sequences shifted one frame from a GxxG or PxxP tetrapeptide in an ECM protein. In particular, the invention relates to GxxG, PxxP, or frame shift active peptides that stimulate production of extracellular matrix proteins and enhance wound closure of the epithelial cell monolayer of scratch-wounded human skin. The peptide compositions may be used in formulations for repairing damaged skin or maintaining healthy skin.

BACKGROUND OF THE INVENTION

Skin aging is commonly viewed as wrinkle formation and impaired wound healing. A wound is defined as a break in the epithelial integrity of the skin. Normal wound healing involves a complex and dynamic but superbly orchestrated series of events leading to the repair of injured tissues. The largest component of normal skin is the extracellular matrix (ECM), a gel-like matrix produced by the cells that it surrounds. The ECM is composed of two major classes including fibrous structural proteins and proteoglycans. Changes in the composition and crosslinked state of the ECM are known to be associated with aging and a range of acquired and heritable skin disorders. It has been well documented that ECM not only provides structural support, but also influences cellular behavior such as differentiation and proliferation. Also, more and more research suggests that the matrix components may be a source of cell signals to facilitate epithelial cell proliferation and migration and thus enhance wound healing.

The largest class of fibrous ECM molecules is the collagen family, which includes at least 16 different types of collagen. Collagen in the dermal matrix is composed primarily of type I (80-85%) and type III (8-11%) collagens, both of which are fibrillar, or rod-shaped, collagens. The tensile strength of skin is due predominately to these fibrillar collagen molecules, which self-assemble into microfibrils in a head-to-tail and staggered side-to-side lateral arrangement. Collagen molecules become cross-linked to adjacent collagen molecules, creating additional strength and stability in collagen fibers. Damage to the collagen network (e.g. by enzymes or physical destruction), or its total collapse causes healing to take place by repair.

Various bioactive peptides that stimulate production of ECM proteins have been reported in both the scientific literature and in issued patents. Peptides historically have been isolated from natural sources and have recently been the subject of structure-function relationship studies. Natural peptides have also served as starting points for the design of synthetic peptide analogs.

Specific sequences within ECM proteins can stimulate useful elements in skin, such as type I collagen, type III collagen, and fibronectin (Katayama et. al., J. Biol. Chem. 288:9941-

9944 (1983)). Katayama et al. identified the pentapeptide, KTTKS (SEQ ID NO:17), within the carboxy-terminal propeptide (residues 197-241) of type I collagen. The propeptide is cleaved during production of the mature collagen protein. The cleaved propeptide may participate in regulating collagen production via a biosynthesis feedback mechanism, with the KTTKS segment playing an active role. Maquart et al. (J Soc Biol. 193:423-28 (1999)) reported that the peptides GHK and CNYYSNS also stimulate ECM synthesis. These sequences may be released during ECM turnover, thereby signaling the need for ECM repair. The short peptide sequences liberated by either mechanism are often called "matrikines" (Maquart et al., J. Soc. Biol. 193:423-28 (1999)).

While a number of natural and synthetic peptides exist, there is a need for improved biologically active peptides and methods for their use.

SUMMARY OF THE INVENTION

Tetrapeptides are disclosed that are characterized by the amino acid sequence motif GxxG or PxxP, where G (glycine) and P (proline) residues are maintained and x is a variable amino acid. The tetrapeptides are derived from sequences that occur multiple times throughout the primary sequence of the ECM protein, type IV collagen. The disclosed sequences induce production of all forms of collagen more than previously known peptide sequences, including KTTKS, sold under the trademark MATRIXYL™ by SEDERMA SAS (France). Further, a composition comprising a combination of various multiply-repeating sequences elicits an even greater collagen-producing response. Additional benefits may be expected from peptide combinations present in a variety of ECM proteins.

Producing a specific combination of tetrapeptides for ECM rebuilding can be commercially cost-prohibitive. A relatively simple and cost-effective means of producing a diverse combination of biologically active tetrapeptides is disclosed. By producing a combinatorial library of tetrapeptides with the GxxG or PxxP motif, a variety of biologically active tetrapeptides can be generated in the same manufacturing run (e.g., GEPG, GPEG, GPPG, and GEEG). The combination of tetrapeptides may induce more formation of ECM proteins than single peptides. Compositions comprising the disclosed tetrapeptides, alone or in combination, are useful in skin care markets including, but not limited to, those that address skin wrinkling, toning, firmness, or sagging. The stimulation of collagen by the disclosed tetrapeptides can significantly improve the health and appearance of damaged and aged skin.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** is SEQ ID NO:45 which is the Collagen IV amino acid sequence illustrating the occurrences of GxxG tetrapeptides. All bold sequences are underlined and overlapping sequences are double-underlined.

FIG. **2** is SEQ ID NO:46 which is the Collagen III amino acid sequence illustrating the occurrences of the frame shift actives PGPR and GAGP. All frame shift active sequences are bold and underlined and the GxxG sequences occurring one frame shift away are double-underlined.

FIG. **3** is also SEQ ID NO:45, the Collagen IV amino acid sequence, illustrating the occurrences of the tetrapeptide PGPP.

DETAILED DESCRIPTION OF THE INVENTION

The invention is generally directed towards tetrapeptides that stimulate production of ECM proteins and modulate wound healing, and uses of such tetrapeptides.

Peptides

One embodiment of the invention is directed towards an isolated tetrapeptide comprising the motif GxxG or PxxP. In this embodiment G (glycine) or P (proline) is maintained and x is a variable amino acid. The peptide can generally be any peptide that falls within the above description, and more preferably is SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12. SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16.

Another embodiment of the invention is directed towards an isolated tetrapeptide comprising the motif GxPG, where x is P at either variable position, or both. In this embodiment, G (glycine) and P (proline) are maintained and x is a variable amino acid. The peptide can generally be any peptide that falls within the above description, and more preferably is SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7.

Another embodiment of the invention is directed towards an isolated tetrapeptide comprising the motif GExG. In this embodiment, G (glycine) and E (glutamic acid) are maintained and x is a variable amino acid. The peptide can generally be any peptide that falls within the above description, and more preferably is SEQ ID NO:5 or SEQ ID NO:8.

Another embodiment of the invention is directed towards an isolated tetrapeptide comprising the motif PGxP. In this embodiment, P (proline) and G (glycine) are maintained and x is a variable amino acid. The peptide can generally be any peptide that falls within the above description, and more preferably is SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:14, or SEQ ID NO:16.

Another embodiment of the invention is directed towards an isolated tetrapeptide comprising the motif PExP. In this embodiment, P (proline) and E (glutamic acid) are maintained and x is a variable amino acid. The peptide can generally be any peptide that falls within the above description, and more preferably is SEQ ID NO:1 or SEQ ID NO:9.

Another embodiment of the invention is directed towards a frame shift active tetrapeptide. In this embodiment, the tetrapeptide occurs one frame shift from either a GxxG or PxxP tetrapeptide in an ECM protein. The peptide can generally be any peptide that falls within the above description, and more preferably is SEQ ID NO:4 or SEQ ID NO:6.

Each of the above-described peptides can comprise D- or L-amino acids. The peptides can comprise all D-amino acids or L-amino acids. The peptides can have an acid C-terminus (—CO_2H) or, preferably, an amide C-terminus (—$CONH_2$, —CONHR, or —$CONR_2$). The peptides may be further augmented or modified, either chemically or enzymatically. For example, the peptides may be amidated (—NH_2) on the C-terminus, which may render the tetrapeptide less susceptible to protease degradation and increase their solubility compared to the free acid forms. The peptides may also be lipidated which may provide for enhanced skin penetration.

The above-described peptides may contain the following amino acids: R (arginine), L (leucine), P (proline), F (phenylalanine), Q (glutamine), E (glutamic acid), I (isoleucine), K (lysine), S (serine), V (valine), A (alanine), N (asparagine), D (aspartic acid), T (threonine), Y (tyrosine) and G (glycine). The above-described peptides do not include the following M (methionine), C (cysteine), H (histidine) or W (tryptophan). Accordingly, in one embodiment, x is not selected from either (methionine), C (cysteine), H (histidine) or W (tryptophan).

Methods of Use

An additional embodiment of the invention is directed towards methods of using the above-described peptides. The methods of use may involve the use of a single peptide, or may involve the use of two or more peptides in combination.

An embodiment of the invention is a method of promoting repair of damaged skin and maintenance of healthy skin using tetrapeptides that stimulate production of ECM proteins. The method generally is directed towards contacting dermal (skin) cells with a composition containing the peptide. The compositions can be an aerosol, emulsion, liquid, lotion, cream, paste, ointment, foam, or other pharmaceutically acceptable formulation. Generally, a pharmaceutically acceptable formulation would include any acceptable carrier suitable for use on human skin, e.g. cosmetically acceptable carrier and dermatological acceptable carrier. The compositions may contain other biologically active agents such as retinoids or other peptides. The compositions may contain pharmaceutically acceptable carriers or adjuvants. The contacting step can be performed in vivo, in situ, in vitro, or by any method known to those of skill in the art. Most preferably, the contacting step is to be performed topically at a concentration sufficient to elicit a stimulatory response. The concentration of the peptide in the composition can be about 0.01 μg/mL to about 100 μg/mL, about 0.1 μg/mL to about 50 μg/mL, and about 0.1 μg/mL to about 1 μg/mL. The contacting step can be performed on a mammal, a cat, a dog, a cow, a horse, a pig, or a human. A preferred composition for promoting ECM protein production comprises SEQ ID NO:8; more preferably, the composition comprises SEQ ID NO:8 in a heterogeneous mixture with at least one other tetrapeptide. In a most preferred embodiment, the individual tetrapeptides in the composition would cause sustained collagen production over a period of at least 48 hours.

An additional embodiment of the invention is directed towards a method for promoting wound healing of skin damaged by normal aging, disease, injury, trauma, or by surgery or other medical procedures. The method can comprise administering to the wound of an animal a composition, wherein the composition comprises any of the above-described peptides, singularly or in combination. The compositions can be a liquid, lotion, cream, paste, ointment, foam, or any other pharmaceutically acceptable formulation. The compositions may contain pharmaceutically acceptable carriers or adjuvants. The compositions may contain other biologically active agents such as antimicrobial agents or growth factors. The compositions may also be used in combination with other therapeutic agents such as tissue grafts, tissue culture products, oxygen or dressings. The concentration of the peptide in the composition can be about 0.01 μg/mL to about 100 μg/mL, about 0.1 μg/mL to about 50 μg/mL, and about 0.1 μg/mL to about 1 μg/mL. The composition can be administered to the wound topically. The animal can generally be any kind of animal, and preferably is a mammal, and more preferably is a human, cow, horse, cat, dog, pig, goat, or sheep. A preferred composition for wound healing applications in which ECM protein production is promoted comprises SEQ ID NO:8; more preferably, the composition comprises SEQ ID NO:8 in a heterogeneous mixture with at least one other tetrapeptide. In a most preferred embodiment, the individual tetrapeptides in the composition would cause sustained collagen production over a period of at least 48 hours.

An additional embodiment of the invention is directed towards a method for reducing scarring of skin damaged by normal aging, disease, injury, trauma, or by surgery or other

medical procedures. The method can comprise administering to the wound of an animal a composition, wherein the composition comprises any of the above-described peptides, singularly or in combination. The compositions can be a liquid, lotion, cream, paste, ointment, foam, or other pharmaceutically acceptable formulation. The compositions may contain pharmaceutically acceptable carriers or adjuvants. The compositions may contain other biologically active agents such as antimicrobial agents or growth factors. The compositions may also be used in combination with other therapeutic agents such as tissue grafts, tissue culture products, oxygen or dressings. The concentration of the peptide in the composition can be about 0.01 μg/mL to about 100 μg/mL, about 0.1 μg/mL to about 50 μg/mL, and about 0.1 μg/mL to about 1 μg/mL. The composition can be administered to the wound topically. The animal can generally be any kind of animal, and preferably is a mammal, and more preferably is a human, cow, horse, cat, dog, pig, goat, or sheep. A preferred composition for wound healing applications in which ECM protein production is promoted comprises SEQ ID NO:8; more preferably, the composition comprises SEQ ID NO:8 in a heterogeneous mixture with at least one other tetrapeptide. In a most preferred embodiment, the individual tetrapeptides in the composition would cause sustained collagen production over a period of at least 48 hours.

A further embodiment of the invention is directed towards a method for producing the disclosed tetrapeptides in combination. The peptides may be produced using any method known to those skilled in the art such as those disclosed in Merrifield, R. B., *Solid Phase Peptide Synthesis I.*, J. Am. Chem. Soc. 85:2149-2154 (1963); Carpino, L. A. et al., [(9-*Fluorenylmethyl)Oxy]Carbonyl (Fmoc) Amino Acid Chlorides: Synthesis, Characterization, And Application To The Rapid Synthesis Of Short Peptides*, J. Org. Chem. 37:51:3732-3734; Merrifield, R. B. et al., *Instrument For Automated Synthesis Of Peptides, Anal.* Chem. 38:1905-1914 (1966); or Kent, S. B. H. et al., *High Yield Chemical Synthesis Of Biologically Active Peptides On An Automated Peptide Synthesizer Of Novel Design*, in: Peptides 1984 (Ragnarsson U., ed.) Almqvist and Wiksell Int., Stockholm (Sweden), pp. 185-188, all of which are incorporated by reference herein in their entirety. Preferably, the peptides will be produced by a machine capable of sequential addition of amino acids to a growing peptide chain. However, the peptides may also be manufactured using standard solution phase methodology.

It has been observed that the addition of a mixture of free amino acids instead of homogenous peptide mixtures during peptide chain synthesis results in varied incorporation of free amino acids such that a combination of peptides results from the synthesis reactions. The relative incorporation frequency of a particular amino acid included in a mixture of two or more amino acids added during synthesis may be adjusted. Adjustment is made possible by modifying the ratio of a free amino acid made available during the synthesis process relative to the other amino acids in the mixture (this is termed an isokinetic mixture).

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLES

Example 1

Identification of Repeat Tetrapeptide Sequences in Collagen

A relatively high proportion of collagen IV tetrapeptide repeat sequences have the motif GxxG (where x is any amino acid). A number of these are shown in situ as part of the full collagen IV sequence illustrated in FIG. **1** as SEQ ID NO:45. Collagen IV was examined first due to its role of interacting with other specialized ECM components (See Gregory Schultz et al., 2005). There are eleven sequences with the GxxG motif in collagen IV that appear more than ten times (GxxG where xx is represented by: vp, ek, fp, lp, pp, sp, ep, ip, pk, qp and tp). Of these tetrapeptide sequences, eight of eleven sequences contain proline in position 3, two of eleven sequences contain P in position 2, one of eleven sequences contains proline in positions 2 and 3, and one of eleven sequences contains no proline. The disclosed sequences are referred to as REPLIKINES™. "REPLIKINE" is defined as a short sequence within ECM proteins that occurs multiple times (i.e., is replicated). This sequence may be present in one ECM protein (e.g., collagen IV). Preferably, the sequence is present in multiple ECM proteins (e.g., all collagens, elastin, laminin, etc.). The presence of the sequence in multiple ECM proteins increases the likelihood that the fragment may be able to promote ECM synthesis or repair.

The eleven GxxG sequences appearing in collagen IV listed above are highlighted in the human collagen IV sequence illustrated in FIG. **1**. In this figure, all bold sequences are underlined and overlapping sequences are double-underlined. All but one of these sequences also appears in collagens I, II, III, and V. This fact contributes to the ability of the disclosed peptides to stimulate the production of all collagen types, particularly when the peptides are used in combination. Table 1 shows the frequency of several tetrapeptide repeats in ECM proteins. Bold sequences in Table 1 are those that appear in collagen IV ten or more times.

TABLE 1

Frequency of tetrapeptides in ECM proteins

SEQ. ID NO	Sequence	Collagen I	Collagen II	Collagen III	Collagen IV	Collagen V	Elastin	Elastin Precursor
19	GAAG	10	5	7		2	4	5
20	GAKG	3	4	3	5	5		
21	GAPG	13	21	25	6	9		

TABLE 1-continued

Frequency of tetrapeptides in ECM proteins									
SEQ. ID NO	Sequence	Collagen I	Collagen II	Collagen III	Collagen IV	Collagen V	Elastin	Elastin Precursor
22	GDKG	2	2	4	9	3		
23	GDRG	2	5	2	4	1		
8	**GEKG**	3	5	4	22	15		
5	**GEPG**	11	15	10	11	4		
24	GERG	10	11	14	6	7		
2	**GFPG**	4	8	6	22	5	1	1
25	GIPG	2	2	6	14	6	5	5
26	GKDG	1	4	5	2	2		
27	GKPG	2	3	3	4	1		
28	GLKG	2	1	1	5	4		
29	GLPG	15	10	9	42	15	1	1
30	GNPG	3	5	3	2	1		
31	GPAG	16	20	20	3	6		
32	GPKG	3	11	4	12	9		
7	**GPPG**	33	40	40	46	43		
33	GPQG	7	11	9	7	5		
34	GPRG	11	13	10	4	7		
35	GPSG	10	11	5	1	5		
36	GPTG	4	3	2	2	6		
37	GPVG	9	3	3	2	5		
38	GQPG	3	4	6	12	7		
39	GRDG	4	2	3	3			
40	GRPG	3	3	4	2	5		
3	**GSPG**	4	6	21	16	3		
41	GTPG	3	4	2	11	2		
42	GVKG	1	3	2	3	1		
43	GVPG		1	3	10	1	14	15
44	GYPG	1	1	1	4	2		

As also evident from a review of the collagen IV sequence, SEQ ID NO:45, there are also many occurrences of sequences having the PxxP motif For example, the sequence PGPP occurs no less than fifteen times as illustrated in FIG. **3**. Therefore, this disclosed sequence is also referred to as a REPLIKINE™. Preferably, this sequence is present in multiple ECM proteins (e.g., all collagens, elastin, laminin, etc.) as the presence of this sequence in multiple ECM proteins increases the likelihood that the fragment may be able to promote ECM synthesis or repair. The fifteen PGPP sequences appearing in collagen IV listed above are highlighted and underlined in the human collagen IV sequence illustrated in FIG. **3**.

Example 2

Identification of Frame Shift Actives

In addition to the relatively high proportion of collagen IV tetrapeptide repeat sequences with the motif GxxG, other tetrapeptide sequences occurring one amino acid frame shift away from a GxxG or PxxP tetrapeptide sequence have been identified. These sequences may repeat or occur only once within an ECM protein and may be located one amino acid position away from either a GxxG or PxxP tetrapeptide sequence as described herein. These tetrapeptide sequences are referred to as frame shift actives. Such frame shift actives may accordingly contain either a G or a P in either the second

or third position depending on the direction of frame shift. It has been further recognized that frame shift actives may be combined with other tetrapeptide sequences disclosed in this application forming a combikine. An example of such a combikine is H06 and H15.

One example of a frame shift active is GAGP or H12 (SEQ ID NO:6). H12 (GAGP) appears one residue (or frame) shift from the GxxG tetrapeptide GGAG in Collagen III (SEQ ID NO:46) as illustrated in FIG. **2**. In this figure, all frame shift active sequences are bold and underlined and the GxxG sequences occurring one frame shift away are double-underlined. Furthermore, as shown in Table 5, this tetrapeptide (GAGP) achieves good results for collagen production at 48 hours. Another example is the sequence PGPR, which is H10 (SEQ ID NO:4) which occurs eleven times in Collagens I-IV. As it appears multiple times in an individual ECM protein, this tetrapeptide would further be considered a REPLIKINE. FIG. **2** (SEQ ID NO:46) illustrates several instances of this tetrapeptide with each occurring one frame shift from the GxxG tetrapeptide GPRG. This particular frame shift active appears in multiple ECM proteins and therefore increases the likelihood that the fragment may be able to promote ECM synthesis or repair.

Example 3

Identification of Repeat Sequences that Stimulate Collagen Production

Several sequences identified in Examples 1 and 2 were synthesized using standard peptide chemistry and assayed for the stimulation of collagen from dermal fibroblasts. The synthesized peptides were amidated at the C-terminus, which rendered the tetrapeptides less susceptible to protease degradation and increased their solubility compared to the free acid forms. Human dermal fibroblasts were incubated in 96-well plates at 37° C. and 5% CO_2 for 24 and 48 hours in 150 μL complete cell culture media (Cascade Biologics, Portland, Oreg.; Cat. No. M-106-500), supplemented with Low Serum Growth Supplement (Cascade Biologics, Portland, Oreg.; Cat. No. S-003-10) containing sample peptides at a final peptide concentration of 50 μg/mL. Each well was seeded with 10,000 cells. Following the incubation, 100-μL medium samples were recovered from each well and assayed for collagen production

The assays were performed by Tebu-bio Laboratories (France) using the SIRCOL™ Collagen Assay Kit (Biocolor Assays, UK) following the manufacturer's protocol. The SIRCOL™ Collagen Assay is a quantitative dye-binding method designed for the analysis of soluble collagens released into culture medium by mammalian cells during in vitro culture. The collagen of the tested samples binds to the anionic SIRCOL™ dye. The collagen-dye complexes precipitate out of solution and are pelleted by centrifugation. The recovered collagen-dye pellet was dissolved in an alkaline solution prior to absorbance measurements. Duplicate measurements were taken at the 24 and 48 hour times from two separate samples. The four measurements for each sample were averaged. The absorbance of reagent blanks, collagen standards, and samples were measured at 560 nm. The reagent blank absorbance was subtracted from the absorbance from each sample at 24 and 48 hours.

Two separate data sets were used to generate two collagen standard calibration curves. The first calibration curve was generated for purposes of calculating the quantity of collagen in samples H6 (combination of SEQ ID NOs:1-4), H7-H14 (SEQ ID NOs:1-8, respectively) and H15 (combination of SEQ ID NOs:5-8). The second calibration curve was generated for calculating the quantity of collagen in samples H16 (SEQ ID NO:9), H21-23 (SEQ ID NOs:10-12, respectively), H25-26 (SEQ ID NOs:13-14, respectively), or H29-30 (SEQ ID NOs:15-16, respectively), H32 (SEQ ID NO:17), H33 (combination of SEQ ID NOs:9-12), H34 (combination of SEQ ID NOs:11-14), H35 (combination of SEQ ID NOs:13-16), H36 (combination of SEQ ID NOs:1, 6, 5, 8), H37 (SEQ ID NO:17) and H38 (SEQ ID NO:8) from the absorbance measurements was created by plotting the $Abs_{560\ nm}$ of the known collagen standards versus the respective concentrations of the collagen standards (in micrograms) each time a series of assays were performed. With respect to each data set, the same calibration curve was used for samples taken at the 24 and 48 hour times (Tables 2A and 2B). Accordingly, different standard curves were prepared immediately prior to performing each series of assays.

TABLE 2A

Calibration curve for assaying collagen production by peptides H6-H15

Collagen standards (μg)	$A_{560\ nm}$ 24 h test	$A_{560\ nm}$ 48 h test
0	0.00	0.00
5	0.08	0.10
10	0.11	0.15
25	0.32	0.35
50	0.66	0.65

TABLE 2B

Calibration curve for assaying collagen production by peptides H16, H21-23, H25-26, and H29-38

Collagen Standards (μg)	$A_{560\ nm}$ Assay date 1	$A_{560\ nm}$ Assay date 2
0	0.00	0.00
5	0.12	0.09
10	0.14	0.15
25	0.48	0.42
50	0.88	0.80

A linear regression was performed from plotting the $Abs_{560\ nm}$ values versus concentrations of the respective collagen standards using MICROSOFT EXCEL™. The regression resulted in a lines described by the formula y=0.013x for both incubation times noted in Table 2A. As the results were identical, only the 24-hour time period was used for the second series calibration curves. The formula of the line obtained on assay date 1 and assay date 2 of the second series of samples was y=0.0178x and y=0.0162x, respectively. The peptide LL-37 (SEQ ID NO:18) was used as a positive control as it has been widely reported to have an impact upon wound healing in man (Heilborn et al., The Cathelicidin Anti-Microbial Peptide LL-37 Is Involved In The Re-Epithelialization Of Human Skin Wounds And Is Lacking In Chronic Ulcer Epithelium, *J. Invest. Dermato.* 120:379-89 (2003)). The assay detection limit defined by the manufacturer is 2.5 μg.

The total amount of collagen produced in samples containing peptides was calculated from the averaged absorbance values taken at 24 hours (Table 3A) and 48 hours (Table 3B)

using the linear equation derived from the standard curve. The total amount of collagen produced in samples containing peptides H16 (SEQ ID NO:9), H21-23 (SEQ ID NOs:10-12, respectively), H25-26 (SEQ ID NOs:13-14, respectively), or H29-30 (SEQ ID NOs:15-16, respectively), H32 (SEQ ID NO:17), H33 (combination of SEQ ID NOs:9-12), H34 (combination of SEQ ID NOs:11-14), H35 (combination of SEQ ID NOs:13-16), H36 (combination of SEQ ID NOs:1, 6, 5, 8), H37 (SEQ ID NO:17) and H38 (SEQ ID NO:8) was calculated from the absorbance values taken at 24 hours (Table 4A) and 48 hours (Table 4B) using the linear equation derived from the standard curve. These values were compared with peptide LL37 (SEQ ID NO:18), a peptide known to stimulate collagen. In each table, samples marked by an asterisk (*) may not be significant as the assay detection limit is 2.5 μg.

TABLE 3A

Absorbance measurements and quantification of collagen in test samples H6-H15 at 24 hours.

SEQ ID NO	Peptides	$A_{560\,nm}$		Average	Average minus blank	Collagen (μg)
18	LL37	0.102	0.136	0.12	0.04	3.0
—	H6	0.084	0.140	0.11	0.03	2.5
1	H7	0.098	0.063	0.08	0.00	0.0*
2	H8	0.122	0.078	0.10	0.02	1.5*
3	H9	0.147	0.104	0.13	0.05	3.5
4	H10	0.103	0.146	0.12	0.04	3.4
5	H11	0.110	0.168	0.14	0.06	4.5
6	H12	0.063	0.101	0.08	0.00	0.2*
7	H13	0.114	0.093	0.10	0.02	1.8*
8	H14	0.115	0.122	0.12	0.04	3.0
—	H15	0.132	0.093	0.11	0.03	2.5
—	Blank	0.074	0.076	0.08	0.00	0.0

TABLE 3B

Absorbance measurements and quantification of collagen in test samples H6-H15 at 48 hours.

SEQ ID NO	Peptides	$A_{560\,nm}$		Average	Average minus blank	Collagen (μg)
18	LL37	0.262	0.113	0.19	0.07	5.2
—	H6	0.086	0.189	0.14	0.02	1.3*
1	H7	0.192	0.189	0.19	0.07	5.4
2	H8	0.137	0.126	0.13	0.01	0.9*
3	H9	0.117	0.061	0.09	0.00	0.0*
4	H10	0.136	0.085	0.11	0.00	0.0*
5	H11	0.113	0.181	0.15	0.03	2.1*
6	H12	0.106	0.231	0.17	0.05	3.7
7	H13	0.100	0.145	0.12	0.00	0.2*
8	H14	0.132	0.176	0.15	0.03	2.6
—	H15	0.177	0.174	0.18	0.06	4.3
—	Blank	0.120	0.115	0.12	0.00	0.0

TABLE 4A

Absorbance measurements and quantification of collagen in test samples H16, H21-23, H25-26, or H29-38 at 24 hours.

SEQ ID NO	Peptides	$A_{560\,nm}$		Average	Average minus blank	Collagen (μg)
9	H16	0.133	0.137	0.14	0.06	3.1
10	H21	0.129	0.119	0.12	0.04	2.5
11	H22	0.192	0.085	0.14	0.06	3.3
12	H23	0.090	0.073	0.08	0.00	0.1*
13	H25	0.129	0.076	0.10	0.02	1.3*

TABLE 4A-continued

Absorbance measurements and quantification of collagen in test samples H16, H21-23, H25-26, or H29-38 at 24 hours.

SEQ ID NO	Peptides	$A_{560\,nm}$		Average	Average minus blank	Collagen (μg)
14	H26	0.114	0.149	0.13	0.05	2.9
15	H29	0.111	0.063	0.09	0.01	0.4*
16	H30	0.099	0.092	0.10	0.02	0.9*
17	H32 (crystals and cell toxicity)	0.087	0.055	0.07	−0.01	−0.5*
—	H33	0.086	0.125	0.11	0.03	1.4*
—	H34	0.117	0.120	0.12	0.04	2.2*
—	H35	0.103	0.090	0.10	0.02	0.9*
—	H36	0.105	0.128	0.12	0.04	2.1*
17	H37	0.099	0.100	0.10	0.02	1.1*
8	H38	0.103	0.159	0.13	0.05	2.9
—	Blank	0.072	0.086	0.08	0.00	0.0

TABLE 4B

Absorbance measurements and quantification of collagen in test samples H16, H21-23, H25-26, or H29-38 at 48 hours.

SEQ ID NO	Peptides	$A_{560\,nm}$		Average	Average minus blank	Collagen (μg)
9	H16	0.065	0.064	0.06	0.00	0.3*
10	H21	0.089	0.126	0.11	0.05	2.9
11	H22	0.102	0.087	0.09	0.03	2.1*
12	H23	0.093	0.082	0.09	0.03	1.7*
13	H25	0.059	0.084	0.07	0.01	0.7*
14	H26	0.081	0.153	0.12	0.06	3.5
15	H29	0.086	0.094	0.09	0.03	1.9*
16	H30	0.083	0.101	0.09	0.03	2.0*
17	H32 (crystals and cell toxicity)	0.088	0.072	0.08	0.02	1.2*
—	H33	0.096	0.092	0.09	0.03	2.1*
—	H34	0.076	0.155	0.12	0.06	3.4
—	H35	0.120	0.074	0.10	0.04	2.3*
—	H36	0.154	0.082	0.12	0.06	3.6
17	H37	0.078	0.114	0.10	0.04	2.2*
8	H38	0.123	0.089	0.11	0.05	2.8
—	Blank	0.106	0.0106	0.06	0.00	0.0

Because sample sizes were 100 μL, the concentration of collagen produced in each sample in micrograms per milliliter is determined by multiplying the amount of collagen detected by ten. The results of all samples tested are summarized in Table 5.

TABLE 5

Collagen synthesis induced by peptides

SEQ ID NO	Name	Primary sequence	[Peptide] (μg/mL).	Collage produced (μg/mL) 24 hrs	48 hrs
1	H07	PEGP	50	0	54
2	H08	GFPG	50	15	9
3	H09	GSPG	50	35	0
4	H10	PGPR	50	34	0
—	H06	H7, H8, H9, H10 (SEQ ID NOs: 1, 2, 3, 4)	50	25	13
5	H11	GEPG	50	45	21
6	H12	GAGP	50	2	37
7	H13	GPPG	50	18	2
8	H14	GEKG	50	30	26
8	H38	GEKG	0.3	29	28
—	H15	H11, H12, H13, H14 (SEQ ID NOs: 5, 6, 7, 8)	50	25	43
9	H16	PEKP	50	31	3
10	H21	PKGP	50	25	29
11	H22	PGQP	50	33	21
12	H23	PGTP	50	1	17
13	H25	PMGP	50	13	7
14	H26	PGPP	50	29	35
15	H29	PQGP	50	4	19
16	H30	PGNP	50	9	20
17	H32	KTTKS (SEDERMA ™ peptide)	50	na	12
17	H37	KTTKS (SEDERMA ™ peptide)	0.3	11	22
—	H33	H16, H21, H22, H23 (SEQ ID NOs: 9, 10, 11, 12)	50	14	21
—	H34	H22, H23, H25, H26 (SEQ ID NOs: 11, 12, 13, 14)	50	22	34
—	H35	H25, H26, H29, H30 (SEQ ID NOs: 13, 14, 15, 16)	50	9	23
—	H36	H7, H12, H11, H14 (SEQ ID NOs: 1, 6, 5, 8)	50	21	36
18	LL37	LLGDFFRKSKEKIGKEFKRIVQRIDFLRNLVPRTES	50	30	52

All tetrapeptides tested stimulated the production of soluble collagen. Of the sequences tested, GxxG tetrapeptides with a glutamic acid in position 2 best stimulate collagen at both 24 and 48 hour time-points. These sequences are H11 (GEPG; SEQ ID NO:5), H14 (GEKG; SEQ ID NO:8) and H38 (GEKG; SEQ ID NO:8). The peptides were initially screened using a peptide concentration of 50 μg/mL. To survey the concentration effective for stimulating collagen production, H14 (SEQ ID NO:8) was also tested at 0.3 μg/mL as H38. As shown in Table 5, H38-induced collagen stimulation was not diminished at the lower concentration, indicating that the maximal stimulating concentration of SEQ ID NO:8 is at or below 0.3 μg/mL.

To test its efficacy, SEQ ID NO:8 (H14 and H38) was compared to the peptide, LL37, (SEQ ID NO:18) which is known to stimulate collagen production. Based on the amount of collagen released by fibroblasts in response to LL37, 25 μg/mL was considered a significant amount of collagen released due to contact with a tetrapeptide. SEQ ID NO:8 induced about the same amount of collagen as LL37 (SEQ ID NO:18) at 24 hours. Importantly, collagen produced as a result of contact with SEQ ID NO:8 was substantially maintained for at least 48 hours. SEQ ID NO:8 was also compared to a leading skin care peptide known to stimulate collagen production, KTTKS (SEQ ID NO:17) (Katayama et. al., J. Biol. Chem. 288:9941-9944 (1983)). KTTKS is an ingredient in the product MATRIXYL™ (SEDERMA SAS, France). SEQ ID NO:8 stimulated more collagen production than the KTTKS (SEQ ID NO:17) peptide (Table 5) at 24 and 48 hours.

Example 4

Identification of Peptide Combinations that Synergistically Enhance Collagen Stimulation—COMBIKINES

Heterogeneous populations of active tetrapeptides may stimulate collagen production at a higher level than homogenous samples of tetrapeptides. The components of the heterogeneous composition are called COMBIKINES™. COMBIKINES are a group of REPLIKINES combined to produce a greater or broader effect upon one or more target cell types. The peptides H11 (SEQ ID NO:5), H12 (SEQ ID NO:6), H13 (SEQ ID NO:7), and H14 (SEQ ID NO:8) were combined to a final concentration of 50 μg/mL and assayed using the same protocol as for the individual peptides. As expected, the result obtained at the 24 hour time point equaled the mean of the individual induction scores. The combination of peptides at 48 hours, however, induced collagen to a level of 43 μg/mL. Surprisingly, this amount was far in excess of the anticipated mean (21 μg/mL) of the four individual peptides (see Table 5). Thus, specific combinations of peptides may stimulate collagen production to a greater degree than the individual peptides at the same concentration. Further, tetrapeptides from a variety of ECM sources such as collagen, laminin, and elastin may produce enhanced induction of a variety of ECM proteins (see Tables 1 and 5).

Example 5

Cost-Effective COMBIKINE Manufacturing for Enhancing Stimulation of Collagen Production

The high cost of peptide synthesis limits the feasibility of producing of heterogeneous compositions of bioactive peptides. The present invention greatly mitigates this limitation.

Because the presently disclosed sequences have a commonality (e.g., a glycine or proline at both termini), a range of tetrapeptides varied at positions 2 and 3 can be synthesized in a single manufacturing run. The synthetic peptides can be made by any method known in the art. (Benoiton, N., *Chemistry of Peptide Synthesis*, CRC (2005)). During manufacture of the peptides, amino acid mixtures are added instead of homogenous samples. The chemistry for determining the correct ratios of amino acid concentrations added at the mixed positions to gain the desired ratio of resulting peptides has been described previously (Greenbaum et al., *Molecular and Cellular Proteomics* 1:60-68, 2002; Krstenansky et al., *Letters in Drug Design and Discovery* 1:6-13, 2004; both of which references are incorporated herein in their entirety). Using this methodology, a library of heterogeneous peptides can be made for nearly the same cost of synthesizing one peptide.

The application of this manufacturing process enables the cost-effective production of bioactive combikines. This is made possible by the unique composition of the disclosed tetrapeptides. The tetrapeptide mixtures are better suited for incorporation into topical use formulations than longer peptides. Because of their length, tetrapeptides have practical and chemical advantages over longer peptides, including the following: easier incorporation and dissolution into formulations, higher skin and pore permeability, and higher production yields with easier methods of manufacturing combinations of peptides. Although not required, the ideal formulations of tetrapeptides, singly or in combination, are formulations that maintain significant collagen production at 24 hours for up to 48 hours. More preferably, the formulations would induce synthesis of ECM for the entire 48 hour period such that more collagen is produced by 48 hours than at 24 hours. Although within the scope of the current invention, tetrapeptides that promote production of ECM proteins at 24 hours, but show diminished production at 48 hours, are less favored. In this regard, Table 6 shows the results of the currently disclosed peptides. Preferred peptides are in bold.

TABLE 6

Disclosed peptides						
SEQ ID NO	Peptides	Released collagen (μg/mL) 24 h	Released collagen (μg/mL) 48 h	Significant release of collagen at 24 h and 48 h	Increase in collagen release at 48 h v. 24 h	Decrease in collagen release at 48 h v. 24 h
18	LL37	30	52	✓	✓	
—	H6	25	13			
1	H7	0	54		✓	
2	H8	15	9			
3	H9	35	0			✓
4	H10	34	0			✓
5	H11	45	21			✓
6	H12	2	37		✓	
7	H13	18	2			
8	**H14**	**30**	**26**	✓		
8	**H38**	**29**	**28**	✓		
—	**H15**	**25**	**43**	✓	✓	
9	H16	31	3			✓
10	**H21**	**25**	**29**	✓		
11	H22	33	21			✓
12	H23	1	17		✓	
13	H25	13	7			✓
14	**H26**	**29**	**35**	✓		
15	H29	4	19		✓	
16	H30	9	20		✓	
17	H32 (crystals and cell toxicity)	NA	12			
17	H37	11	22		✓	
—	H33	14	21		✓	
—	H34	22	34		✓	
—	H35	9	23		✓	
—	H36	21	36		✓	

Example 6

Collagen Stimulators Also Serve as Multi-Effector Molecules Enhancing Skin Epithelial Cell Wound Closer

Collagens are key components of all phases of wound healing. Stimulation of collagen production reflects that damage has occurred to the collagen network (e.g. by enzymes or physical destruction). Indeed, the total collapse of the collagen network in fact causes healing to take place. Therefore a collagen stimulator may also serve as a multi-effector molecule orchestrating certain matrix remodeling and enhancing wound healing.

Wound healing experiments were performed on monolayers of human skin epithelial cells (CRL-2592) plated onto 12-well plates. Cells were serum-starved for 24 hours before experimentation. Confluent monolayers of CRL-2592 were wounded using a P200 (200-μL) pipette tip. The wounds were washed and picture-documented prior to peptide treatment. Peptides were added to a final concentration from 20 to 40 μg/ml. Cells were kept in an incubator at 37° C., 5% CO_2, and 92% humidity, except when images were being captured for a short period at room temperature. Wound closure was followed at 6-hour and 10-hour time points. PBS-treated wounds were used as negative controls for comparison purposes.

TABLE 7

Effect of peptides on human skin epithelial wound closure in vitro

	0 hr	6 hr		10 hr	
Compound	W-size*	W-size	% closure	W-size	% closure
PBS-1	36	29	19.40%	21	41.70%
PBS-2	52	42	19.20%	30	42.30%
SEQ ID NO: 14	25	12	52%	2.75	89%
SEQ ID NO: 5	48	39	19%	30	37.50%

*W-size: wound size (arbitrary)

In vitro monolayer wound closure is a result of cell migration, which is important in many biological processes such as embryogenesis, angiogenesis, inflammatory reactions and wound repair. These processes are thought to be regulated by interactions with other cells, cytokines and ECM proteins. As shown in Table 7, SEQ ID NO:14 significantly induces wound closure compared to the effects of PBS alone. Such activity is peptide-specific as well as cell type-specific since SEQ ID NO:14 does not induce wound closure in a human skin fibroblast monolayer (data not shown). SEQ ID NO:5 is also a collagen inducer, but does not enhance wound closure or epithelial cell migration to any great extent compared to the effects of PBS alone. The fact that SEQ ID NO:14 induced cell migration or wound closure in a manner specific to skin epithelial cells (i.e. does not recruit fibroblasts) may add an advantage to using this peptide for skin care, since it is believed that the recruitment of large numbers of active fibroblasts to a wound site results in excess deposition and contraction of tissue resulting in scarring.

All of the compositions or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention.

SEQUENCE LISTING

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Lys Thr Thr Lys Ser
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Phe Lys Arg Ile Val Gln Arg Ile Asp Phe Leu Arg Asn Leu Val Pro
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Arg Thr Glu Ser
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Gly Leu Lys Gly
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Gly Gln Pro Gly
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Gly Arg Asp Gly
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Met Gly Pro Arg Leu Ser Val Trp Leu Leu Leu Leu Pro Ala Ala Leu
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Leu Leu His Glu Glu His Ser Arg Ala Ala Ala Lys Gly Gly Cys Ala
20 25 30

Gly Ser Gly Cys Gly Lys Cys Asp Cys His Gly Val Lys Gly Gln Lys
35 40 45

Gly Glu Arg Gly Leu Pro Gly Leu Gln Gly Val Ile Gly Phe Pro Gly
50 55 60

Met Gln Gly Pro Glu Gly Pro Gln Gly Pro Pro Gly Gln Lys Gly Asp
65 70 75 80

Thr Gly Glu Pro Gly Leu Pro Gly Thr Lys Gly Thr Arg Gly Pro Pro
85 90 95

Gly Ala Ser Gly Tyr Pro Gly Asn Pro Gly Leu Pro Gly Ile Pro Gly
100 105 110

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Gln Asp Gly Pro Pro Gly Pro Pro Gly Ile Pro Gly Cys Asn Gly Thr
        115                 120                 125

Lys Gly Glu Arg Gly Pro Leu Gly Pro Pro Gly Leu Pro Gly Phe Ala
    130                 135                 140

Gly Asn Pro Gly Pro Pro Gly Leu Pro Gly Met Lys Gly Asp Pro Gly
145                 150                 155                 160

Glu Ile Leu Gly His Val Pro Gly Met Leu Leu Lys Gly Glu Arg Gly
                165                 170                 175

Phe Pro Gly Ile Pro Gly Thr Pro Gly Pro Pro Gly Leu Pro Gly Leu
            180                 185                 190

Gln Gly Pro Val Gly Pro Pro Gly Phe Thr Gly Pro Pro Gly Pro Pro
        195                 200                 205

Gly Pro Pro Gly Pro Pro Gly Glu Lys Gly Gln Met Gly Leu Ser Phe
    210                 215                 220

Gln Gly Pro Lys Gly Asp Lys Gly Asp Gln Gly Val Ser Gly Pro Pro
225                 230                 235                 240

Gly Val Pro Gly Gln Ala Gln Val Gln Glu Lys Gly Asp Phe Ala Thr
                245                 250                 255

Lys Gly Glu Lys Gly Gln Lys Gly Glu Pro Gly Phe Gln Gly Met Pro
            260                 265                 270

Gly Val Gly Glu Lys Gly Glu Pro Gly Lys Pro Gly Pro Arg Gly Lys
        275                 280                 285

Pro Gly Lys Asp Gly Asp Lys Gly Glu Lys Gly Ser Pro Gly Phe Pro
    290                 295                 300

Gly Glu Pro Gly Tyr Pro Gly Leu Ile Gly Arg Gln Gly Pro Gln Gly
305                 310                 315                 320

Glu Lys Gly Glu Ala Gly Pro Pro Gly Pro Pro Gly Ile Val Ile Gly
                325                 330                 335

Thr Gly Pro Leu Gly Glu Lys Gly Glu Arg Gly Tyr Pro Gly Thr Pro
            340                 345                 350

Gly Pro Arg Gly Glu Pro Gly Pro Lys Gly Phe Pro Gly Leu Pro Gly
        355                 360                 365

Gln Pro Gly Pro Pro Gly Leu Pro Val Pro Gly Gln Ala Gly Ala Pro
    370                 375                 380

Gly Phe Pro Gly Glu Arg Gly Glu Lys Gly Asp Arg Gly Phe Pro Gly
385                 390                 395                 400

Thr Ser Leu Pro Gly Pro Ser Gly Arg Asp Gly Leu Pro Gly Pro Pro
                405                 410                 415

Gly Ser Pro Gly Pro Pro Gly Gln Pro Gly Tyr Thr Asn Gly Ile Val
            420                 425                 430

Glu Cys Gln Pro Gly Pro Pro Gly Asp Gln Gly Pro Pro Gly Ile Pro
        435                 440                 445

Gly Gln Pro Gly Phe Ile Gly Glu Ile Gly Glu Lys Gly Gln Lys Gly
    450                 455                 460

Glu Ser Cys Leu Ile Cys Asp Ile Asp Gly Tyr Arg Gly Pro Pro Gly
465                 470                 475                 480

Pro Gln Gly Pro Pro Gly Glu Ile Gly Phe Pro Gly Gln Pro Gly Ala
                485                 490                 495

Lys Gly Asp Arg Gly Leu Pro Gly Arg Asp Gly Val Ala Gly Val Pro
            500                 505                 510

Gly Pro Gln Gly Thr Pro Gly Leu Ile Gly Gln Pro Gly Ala Lys Gly
        515                 520                 525

Glu Pro Gly Glu Phe Tyr Phe Asp Leu Arg Leu Lys Gly Asp Lys Gly
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530 535 540

Asp Pro Gly Phe Pro Gly Gln Pro Gly Met Pro Gly Arg Ala Gly Ser
545 550 555 560

Pro Gly Arg Asp Gly His Pro Gly Leu Pro Gly Pro Lys Gly Ser Pro
565 570 575

Gly Ser Val Gly Leu Lys Gly Glu Arg Gly Pro Pro Gly Gly Val Gly
580 585 590

Phe Pro Gly Ser Arg Gly Asp Thr Gly Pro Pro Gly Pro Pro Gly Tyr
595 600 605

Gly Pro Ala Gly Pro Ile Gly Asp Lys Gly Gln Ala Gly Phe Pro Gly
610 615 620

Gly Pro Gly Ser Pro Gly Leu Pro Gly Pro Lys Gly Glu Pro Gly Lys
625 630 635 640

Ile Val Pro Leu Pro Gly Pro Pro Gly Ala Glu Gly Leu Pro Gly Ser
645 650 655

Pro Gly Phe Pro Gly Pro Gln Gly Asp Arg Gly Phe Pro Gly Thr Pro
660 665 670

Gly Arg Pro Gly Leu Pro Gly Glu Lys Gly Ala Val Gly Gln Pro Gly
675 680 685

Ile Gly Phe Pro Gly Pro Pro Gly Pro Lys Gly Val Asp Gly Leu Pro
690 695 700

Gly Asp Met Gly Pro Pro Gly Thr Pro Gly Arg Pro Gly Phe Asn Gly
705 710 715 720

Leu Pro Gly Asn Pro Gly Val Gln Gly Gln Lys Gly Glu Pro Gly Val
725 730 735

Gly Leu Pro Gly Leu Lys Gly Leu Pro Gly Leu Pro Gly Ile Pro Gly
740 745 750

Thr Pro Gly Glu Lys Gly Ser Ile Gly Val Pro Gly Val Pro Gly Glu
755 760 765

His Gly Ala Ile Gly Pro Pro Gly Leu Gln Gly Ile Arg Gly Glu Pro
770 775 780

Gly Pro Pro Gly Leu Pro Gly Ser Val Gly Ser Pro Gly Val Pro Gly
785 790 795 800

Ile Gly Pro Pro Gly Ala Arg Gly Pro Pro Gly Gly Gln Gly Pro Pro
805 810 815

Gly Leu Ser Gly Pro Pro Gly Ile Lys Gly Glu Lys Gly Phe Pro Gly
820 825 830

Phe Pro Gly Leu Asp Met Pro Gly Pro Lys Gly Asp Lys Gly Ala Gln
835 840 845

Gly Leu Pro Gly Ile Thr Gly Gln Ser Gly Leu Pro Gly Leu Pro Gly
850 855 860

Gln Gln Gly Ala Pro Gly Ile Pro Gly Phe Pro Gly Ser Lys Gly Glu
865 870 875 880

Met Gly Val Met Gly Thr Pro Gly Gln Pro Gly Ser Pro Gly Pro Val
885 890 895

Gly Ala Pro Gly Leu Pro Gly Glu Lys Gly Asp His Gly Phe Pro Gly
900 905 910

Ser Ser Gly Pro Arg Gly Asp Pro Gly Leu Lys Gly Asp Lys Gly Asp
915 920 925

Val Gly Leu Pro Gly Lys Pro Gly Ser Met Asp Lys Val Asp Met Gly
930 935 940

Ser Met Lys Gly Gln Lys Gly Asp Gln Gly Glu Lys Gly Gln Ile Gly
945 950 955 960

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Pro Ile Gly Glu Lys Gly Ser Arg Gly Asp Pro Gly Thr Pro Gly Val
               965                 970                 975

Pro Gly Lys Asp Gly Gln Ala Gly Gln Pro Gly Gln Pro Gly Pro Lys
            980                 985                 990

Gly Asp Pro Gly Ile Ser Gly Thr  Pro Gly Ala Pro Gly  Leu Pro Gly
        995                 1000                1005

Pro Lys  Gly Ser Val Gly Gly  Met Gly Leu Pro Gly  Thr Pro Gly
    1010                1015                1020

Glu Lys  Gly Val Pro Gly Ile  Pro Gly Pro Gln Gly  Ser Pro Gly
    1025                1030                1035

Leu Pro  Gly Asp Lys Gly Ala  Lys Gly Glu Lys Gly  Gln Ala Gly
    1040                1045                1050

Pro Pro  Gly Ile Gly Ile Pro  Gly Leu Arg Gly Glu  Lys Gly Asp
    1055                1060                1065

Gln Gly  Ile Ala Gly Phe Pro  Gly Ser Pro Gly Glu  Lys Gly Glu
    1070                1075                1080

Lys Gly  Ser Ile Gly Ile Pro  Gly Met Pro Gly Ser  Pro Gly Leu
    1085                1090                1095

Lys Gly  Ser Pro Gly Ser Val  Gly Tyr Pro Gly Ser  Pro Gly Leu
    1100                1105                1110

Pro Gly  Glu Lys Gly Asp Lys  Gly Leu Pro Gly Leu  Asp Gly Ile
    1115                1120                1125

Pro Gly  Val Lys Gly Glu Ala  Gly Leu Pro Gly Thr  Pro Gly Pro
    1130                1135                1140

Thr Gly  Pro Ala Gly Gln Lys  Gly Glu Pro Gly Ser  Asp Gly Ile
    1145                1150                1155

Pro Gly  Ser Ala Gly Glu Lys  Gly Glu Pro Gly Leu  Pro Gly Arg
    1160                1165                1170

Gly Phe  Pro Gly Phe Pro Gly  Ala Lys Gly Asp Lys  Gly Ser Lys
    1175                1180                1185

Gly Glu  Val Gly Phe Pro Gly  Leu Ala Gly Ser Pro  Gly Ile Pro
    1190                1195                1200

Gly Ser  Lys Gly Glu Gln Gly  Phe Met Gly Pro Pro  Gly Pro Gln
    1205                1210                1215

Gly Gln  Pro Gly Leu Pro Gly  Ser Pro Gly His Ala  Thr Glu Gly
    1220                1225                1230

Pro Lys  Gly Asp Arg Gly Pro  Gln Gly Gln Pro Gly  Leu Pro Gly
    1235                1240                1245

Leu Pro  Gly Pro Met Gly Pro  Pro Gly Leu Pro Gly  Ile Asp Gly
    1250                1255                1260

Val Lys  Gly Asp Lys Gly Asn  Pro Gly Trp Pro Gly  Ala Pro Gly
    1265                1270                1275

Val Pro  Gly Pro Lys Gly Asp  Pro Gly Phe Gln Gly  Met Pro Gly
    1280                1285                1290

Ile Gly  Gly Ser Pro Gly Ile  Thr Gly Ser Lys Gly  Asp Met Gly
    1295                1300                1305

Pro Pro  Gly Val Pro Gly Phe  Gln Gly Pro Lys Gly  Leu Pro Gly
    1310                1315                1320

Leu Gln  Gly Ile Lys Gly Asp  Gln Gly Asp Gln Gly  Val Pro Gly
    1325                1330                1335

Ala Lys  Gly Leu Pro Gly Pro  Pro Gly Pro Pro Gly  Pro Tyr Asp
    1340                1345                1350

Ile Ile  Lys Gly Glu Pro Gly  Leu Pro Gly Pro Glu  Gly Pro Pro
    1355                1360                1365
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Gly Leu  Lys Gly Leu Gln Gly  Leu Pro Gly Pro Lys  Gly Gln Gln
    1370                 1375                 1380

Gly Val  Thr Gly Leu Val Gly  Ile Pro Gly Pro Pro  Gly Ile Pro
    1385                 1390                 1395

Gly Phe  Asp Gly Ala Pro Gly  Gln Lys Gly Glu Met  Gly Pro Ala
    1400                 1405                 1410

Gly Pro  Thr Gly Pro Arg Gly  Phe Pro Gly Pro Pro  Gly Pro Asp
    1415                 1420                 1425

Gly Leu  Pro Gly Ser Met Gly  Pro Pro Gly Thr Pro  Ser Val Asp
    1430                 1435                 1440

His Gly  Phe Leu Val Thr Arg  His Ser Gln Thr Ile  Asp Asp Pro
    1445                 1450                 1455

Gln Cys  Pro Ser Gly Thr Lys  Ile Leu Tyr His Gly  Tyr Ser Leu
    1460                 1465                 1470

Leu Tyr  Val Gln Gly Asn Glu  Arg Ala His Gly Gln  Asp Leu Gly
    1475                 1480                 1485

Thr Ala  Gly Ser Cys Leu Arg  Lys Phe Ser Thr Met  Pro Phe Leu
    1490                 1495                 1500

Phe Cys  Asn Ile Asn Asn Val  Cys Asn Phe Ala Ser  Arg Asn Asp
    1505                 1510                 1515

Tyr Ser  Tyr Trp Leu Ser Thr  Pro Glu Pro Met Pro  Met Ser Met
    1520                 1525                 1530

Ala Pro  Ile Thr Gly Glu Asn  Ile Arg Pro Phe Ile  Ser Arg Cys
    1535                 1540                 1545

Ala Val  Cys Glu Ala Pro Ala  Met Val Met Ala Val  His Ser Gln
    1550                 1555                 1560

Thr Ile  Gln Ile Pro Pro Cys  Pro Ser Gly Trp Ser  Ser Leu Trp
    1565                 1570                 1575

Ile Gly  Tyr Ser Phe Val Met  His Thr Ser Ala Gly  Ala Glu Gly
    1580                 1585                 1590

Ser Gly  Gln Ala Leu Ala Ser  Pro Gly Ser Cys Leu  Glu Glu Phe
    1595                 1600                 1605

Arg Ser  Ala Pro Phe Ile Glu  Cys His Gly Arg Gly  Thr Cys Asn
    1610                 1615                 1620

Tyr Tyr  Ala Asn Ala Tyr Ser  Phe Trp Leu Ala Thr  Ile Glu Arg
    1625                 1630                 1635

Ser Glu  Met Phe Lys Lys Pro  Thr Pro Ser Thr Leu  Lys Ala Gly
    1640                 1645                 1650

Glu Leu  Arg Thr His Val Ser  Arg Cys Gln Val Cys  Met Arg Arg
    1655                 1660                 1665

Thr
```

<210> SEQ ID NO 46
<211> LENGTH: 1466
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 46

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Met Met Ser Phe Val Gln Lys Gly Ser Trp Leu Leu Leu Ala Leu Leu
1               5                  10                  15

His Pro Thr Ile Ile Leu Ala Gln Gln Glu Ala Val Glu Gly Gly Cys
            20                  25                  30

Ser His Leu Gly Gln Ser Tyr Ala Asp Arg Asp Val Trp Lys Pro Glu
        35                  40                  45
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Pro Cys Gln Ile Cys Val Cys Asp Ser Gly Ser Val Leu Cys Asp Asp
    50                  55                  60

Ile Ile Cys Asp Asp Gln Glu Leu Asp Cys Pro Asn Pro Glu Ile Pro
65                  70                  75                  80

Phe Gly Glu Cys Cys Ala Val Cys Pro Gln Pro Pro Thr Ala Pro Thr
                85                  90                  95

Arg Pro Pro Asn Gly Gln Gly Pro Gln Gly Pro Lys Gly Asp Pro Gly
            100                 105                 110

Pro Pro Gly Ile Pro Gly Arg Asn Gly Asp Pro Gly Ile Pro Gly Gln
        115                 120                 125

Pro Gly Ser Pro Gly Ser Pro Gly Pro Pro Gly Ile Cys Glu Ser Cys
    130                 135                 140

Pro Thr Gly Pro Gln Asn Tyr Ser Pro Gln Tyr Asp Ser Tyr Asp Val
145                 150                 155                 160

Lys Ser Gly Val Ala Val Gly Gly Leu Ala Gly Tyr Pro Gly Pro Ala
                165                 170                 175

Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Thr Ser Gly His Pro Gly
            180                 185                 190

Ser Pro Gly Ser Pro Gly Tyr Gln Gly Pro Pro Gly Glu Pro Gly Gln
        195                 200                 205

Ala Gly Pro Ser Gly Pro Pro Gly Pro Pro Gly Ala Ile Gly Pro Ser
    210                 215                 220

Gly Pro Ala Gly Lys Asp Gly Glu Ser Gly Arg Pro Gly Arg Pro Gly
225                 230                 235                 240

Glu Arg Gly Leu Pro Gly Pro Pro Gly Ile Lys Gly Pro Ala Gly Ile
                245                 250                 255

Pro Gly Phe Pro Gly Met Lys Gly His Arg Gly Phe Asp Gly Arg Asn
            260                 265                 270

Gly Glu Lys Gly Glu Thr Gly Ala Pro Gly Leu Lys Gly Glu Asn Gly
        275                 280                 285

Leu Pro Gly Glu Asn Gly Ala Pro Gly Pro Met Gly Pro Arg Gly Ala
    290                 295                 300

Pro Gly Glu Arg Gly Arg Pro Gly Leu Pro Gly Ala Ala Gly Ala Arg
305                 310                 315                 320

Gly Asn Asp Gly Ala Arg Gly Ser Asp Gly Gln Pro Gly Pro Pro Gly
                325                 330                 335

Pro Pro Gly Thr Ala Gly Phe Pro Gly Ser Pro Gly Ala Lys Gly Glu
            340                 345                 350

Val Gly Pro Ala Gly Ser Pro Gly Ser Asn Gly Ala Pro Gly Gln Arg
        355                 360                 365

Gly Glu Pro Gly Pro Gln Gly His Ala Gly Ala Gln Gly Pro Pro Gly
    370                 375                 380

Pro Pro Gly Ile Asn Gly Ser Pro Gly Gly Lys Gly Glu Met Gly Pro
385                 390                 395                 400

Ala Gly Ile Pro Gly Ala Pro Gly Leu Met Gly Ala Arg Gly Pro Pro
                405                 410                 415

Gly Pro Ala Gly Ala Asn Gly Ala Pro Gly Leu Arg Gly Gly Ala Gly
            420                 425                 430

Glu Pro Gly Lys Asn Gly Ala Lys Gly Glu Pro Gly Pro Arg Gly Glu
        435                 440                 445

Arg Gly Glu Ala Gly Ile Pro Gly Val Pro Gly Ala Lys Gly Glu Asp
    450                 455                 460

Gly Lys Asp Gly Ser Pro Gly Glu Pro Gly Ala Asn Gly Leu Pro Gly
465                 470                 475                 480
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Ala Ala Gly Glu Arg Gly Ala Pro Gly Phe Arg Gly Pro Ala Gly Pro
                485                 490                 495

Asn Gly Ile Pro Gly Glu Lys Gly Pro Ala Gly Glu Arg Gly Ala Pro
            500                 505                 510

Gly Pro Ala Gly Pro Arg Gly Ala Ala Gly Glu Pro Gly Arg Asp Gly
        515                 520                 525

Val Pro Gly Gly Pro Gly Met Arg Gly Met Pro Gly Ser Pro Gly Gly
    530                 535                 540

Pro Gly Ser Asp Gly Lys Pro Gly Pro Pro Gly Ser Gln Gly Glu Ser
545                 550                 555                 560

Gly Arg Pro Gly Pro Pro Gly Pro Ser Gly Pro Arg Gly Gln Pro Gly
                565                 570                 575

Val Met Gly Phe Pro Gly Pro Lys Gly Asn Asp Gly Ala Pro Gly Lys
            580                 585                 590

Asn Gly Glu Arg Gly Gly Pro Gly Gly Pro Gly Pro Gln Gly Pro Pro
        595                 600                 605

Gly Lys Asn Gly Glu Thr Gly Pro Gln Gly Pro Pro Gly Pro Thr Gly
    610                 615                 620

Pro Gly Gly Asp Lys Gly Asp Thr Gly Pro Pro Gly Pro Gln Gly Leu
625                 630                 635                 640

Gln Gly Leu Pro Gly Thr Gly Gly Pro Pro Gly Glu Asn Gly Lys Pro
                645                 650                 655

Gly Glu Pro Gly Pro Lys Gly Asp Ala Gly Ala Pro Gly Ala Pro Gly
            660                 665                 670

Gly Lys Gly Asp Ala Gly Ala Pro Gly Glu Arg Gly Pro Pro Gly Leu
        675                 680                 685

Ala Gly Ala Pro Gly Leu Arg Gly Gly Ala Gly Pro Pro Gly Pro Glu
    690                 695                 700

Gly Gly Lys Gly Ala Ala Gly Pro Pro Gly Pro Pro Gly Ala Ala Gly
705                 710                 715                 720

Thr Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly Gly Leu Gly Ser
                725                 730                 735

Pro Gly Pro Lys Gly Asp Lys Gly Glu Pro Gly Gly Pro Gly Ala Asp
            740                 745                 750

Gly Val Pro Gly Lys Asp Gly Pro Arg Gly Pro Thr Gly Pro Ile Gly
        755                 760                 765

Pro Pro Gly Pro Ala Gly Gln Pro Gly Asp Lys Gly Glu Gly Gly Ala
    770                 775                 780

Pro Gly Leu Pro Gly Ile Ala Gly Pro Arg Gly Ser Pro Gly Glu Arg
785                 790                 795                 800

Gly Glu Thr Gly Pro Pro Gly Pro Ala Gly Phe Pro Gly Ala Pro Gly
                805                 810                 815

Gln Asn Gly Glu Pro Gly Gly Lys Gly Glu Arg Gly Ala Pro Gly Glu
            820                 825                 830

Lys Gly Glu Gly Gly Pro Pro Gly Val Ala Gly Pro Pro Gly Gly Ser
        835                 840                 845

Gly Pro Ala Gly Pro Pro Gly Pro Gln Gly Val Lys Gly Glu Arg Gly
    850                 855                 860

Ser Pro Gly Gly Pro Gly Ala Ala Gly Phe Pro Gly Ala Arg Gly Leu
865                 870                 875                 880

Pro Gly Pro Pro Gly Ser Asn Gly Asn Pro Gly Pro Pro Gly Pro Ser
                885                 890                 895

Gly Ser Pro Gly Lys Asp Gly Pro Pro Gly Pro Ala Gly Asn Thr Gly
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            900                 905                 910

Ala Pro Gly Ser Pro Gly Val Ser Gly Pro Lys Gly Asp Ala Gly Gln
        915                 920                 925

Pro Gly Glu Lys Gly Ser Pro Gly Ala Gln Gly Pro Pro Gly Ala Pro
    930                 935                 940

Gly Pro Leu Gly Ile Ala Gly Ile Thr Gly Ala Arg Gly Leu Ala Gly
945                 950                 955                 960

Pro Pro Gly Met Pro Gly Pro Arg Gly Ser Pro Gly Pro Gln Gly Val
                965                 970                 975

Lys Gly Glu Ser Gly Lys Pro Gly Ala Asn Gly Leu Ser Gly Glu Arg
            980                 985                 990

Gly Pro Pro Gly Pro Gln Gly Leu  Pro Gly Leu Ala Gly  Thr Ala Gly
        995                 1000                1005

Glu Pro  Gly Arg Asp Gly Asn  Pro Gly Ser Asp Gly  Leu Pro Gly
    1010                1015                1020

Arg Asp  Gly Ser Pro Gly Gly  Lys Gly Asp Arg Gly  Glu Asn Gly
    1025                1030                1035

Ser Pro  Gly Ala Pro Gly Ala  Pro Gly His Pro Gly  Pro Pro Gly
    1040                1045                1050

Pro Val  Gly Pro Ala Gly Lys  Ser Gly Asp Arg Gly  Glu Ser Gly
    1055                1060                1065

Pro Ala  Gly Pro Ala Gly Ala  Pro Gly Pro Ala Gly  Ser Arg Gly
    1070                1075                1080

Ala Pro  Gly Pro Gln Gly Pro  Arg Gly Asp Lys Gly  Glu Thr Gly
    1085                1090                1095

Glu Arg  Gly Ala Ala Gly Ile  Lys Gly His Arg Gly  Phe Pro Gly
    1100                1105                1110

Asn Pro  Gly Ala Pro Gly Ser  Pro Gly Pro Ala Gly  Gln Gln Gly
    1115                1120                1125

Ala Ile  Gly Ser Pro Gly Pro  Ala Gly Pro Arg Gly  Pro Val Gly
    1130                1135                1140

Pro Ser  Gly Pro Pro Gly Lys  Asp Gly Thr Ser Gly  His Pro Gly
    1145                1150                1155

Pro Ile  Gly Pro Pro Gly Pro  Arg Gly Asn Arg Gly  Glu Arg Gly
    1160                1165                1170

Ser Glu  Gly Ser Pro Gly His  Pro Gly Gln Pro Gly  Pro Pro Gly
    1175                1180                1185

Pro Pro  Gly Ala Pro Gly Pro  Cys Cys Gly Gly Val  Gly Ala Ala
    1190                1195                1200

Ala Ile  Ala Gly Ile Gly Gly  Glu Lys Ala Gly Gly  Phe Ala Pro
    1205                1210                1215

Tyr Tyr  Gly Asp Glu Pro Met  Asp Phe Lys Ile Asn  Thr Asp Glu
    1220                1225                1230

Ile Met  Thr Ser Leu Lys Ser  Val Asn Gly Gln Ile  Glu Ser Leu
    1235                1240                1245

Ile Ser  Pro Asp Gly Ser Arg  Lys Asn Pro Ala Arg  Asn Cys Arg
    1250                1255                1260

Asp Leu  Lys Phe Cys His Pro  Glu Leu Lys Ser Gly  Glu Tyr Trp
    1265                1270                1275

Val Asp  Pro Asn Gln Gly Cys  Lys Leu Asp Ala Ile  Lys Val Phe
    1280                1285                1290

Cys Asn  Met Glu Thr Gly Glu  Thr Cys Ile Ser Ala  Asn Pro Leu
    1295                1300                1305
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Asn Val  Pro Arg Lys His Trp  Trp Thr Asp Ser Ser  Ala Glu Lys
    1310                 1315                 1320

Lys His  Val Trp Phe Gly Glu  Ser Met Asp Gly Gly  Phe Gln Phe
    1325                 1330                 1335

Ser Tyr  Gly Asn Pro Glu Leu  Pro Glu Asp Val Leu  Asp Val Gln
    1340                 1345                 1350

Leu Ala  Phe Leu Arg Leu Leu  Ser Ser Arg Ala Ser  Gln Asn Ile
    1355                 1360                 1365

Thr Tyr  His Cys Lys Asn Ser  Ile Ala Tyr Met Asp  Gln Ala Ser
    1370                 1375                 1380

Gly Asn  Val Lys Lys Ala Leu  Lys Leu Met Gly Ser  Asn Glu Gly
    1385                 1390                 1395

Glu Phe  Lys Ala Glu Gly Asn  Ser Lys Phe Thr Tyr  Thr Val Leu
    1400                 1405                 1410

Glu Asp  Gly Cys Thr Lys His  Thr Gly Glu Trp Ser  Lys Thr Val
    1415                 1420                 1425

Phe Glu  Tyr Arg Thr Arg Lys  Ala Val Arg Leu Pro  Ile Val Asp
    1430                 1435                 1440

Ile Ala  Pro Tyr Asp Ile Gly  Gly Pro Asp Gln Glu  Phe Gly Val
    1445                 1450                 1455

Asp Val  Gly Pro Val Cys Phe  Leu
    1460                 1465
```

What is claimed is:

1. A tetrapeptide capable of inducing production of extracellular matrix proteins, wherein the amino acid sequence of the tetrapeptide consists of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:16 having an acid or amidated carboxy-terminus.

2. A composition comprising at least one tetrapeptide of claim **1** and a pharmaceutically acceptable carrier.

3. The composition of claim **2**, wherein the tetrapeptide is present in a concentration ranging from about 0.1 μg/mL to about 50 μg/mL.

4. The composition of claim **2**, wherein the composition is in the form of an aerosol, emulsion, liquid, lotion, cream, paste, ointment, or foam.

5. A method for stimulating the production of collagen in a human in need thereof, the method comprising administering to said human a therapeutically effective amount of the composition of claim **2**.

6. The method of claim **5**, wherein the therapeutically effective concentration is in the range of about 0.1 μg/mL to about 50 μg/mL of tetrapeptide.

7. The method of claim **5**, wherein the administering to said human a therapeutically effective amount of the composition promotes wound healing of damaged skin.

8. The tetrapeptide of claim **1**, wherein the tetrapeptide is SEQ ID NO:10.

9. The tetrapeptide of claim **1**, wherein the tetrapeptide is SEQ ID NO:9.

10. The method of claim **7**, wherein said damaged skin is a result of aging, disease, injury, trauma, or surgery.

11. The composition of claim **2** which further comprises a retinoid.

12. The method of claim **7** wherein the composition is administered topically to a site of damaged skin.

13. A skincare composition comprising at least one tetrapeptide of claim **1** and a dermatological acceptable carrier.

14. The skincare composition of claim **13**, wherein the composition is in the form of an aerosol, emulsion, lotion, cream, paste, ointment, or foam.

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